Asian J. Pharm. Res. Vol 4, Issue 3, 134-140, 2014.

e-ISSN 2231 – 363X
Print ISSN 2231 – 3621

Asian Journal
of
PHARMACEUTICAL RESEARCH
Journal homepage: - www.ajprjournal.com

ANALYTICAL METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF SAXAGLIPTIN AND METHYLDOPA

R.L. Sawant, S.M. Mhaske*

Department of Pharmaceutical Chemistry and PG Studies, Padmashree Dr. Vithalrao Vikhe Patil Foundation's, College of Pharmacy, Vilad Ghat, Ahmednagar-414111,India.

ABSTRACT

Four simple, accurate and precise spectrophotometric methods have been developed for simultaneous determination of saxagliptin (SAXA) and methyldopa (MD) in a laboratory mixture. Simultaneous equation method (Method I) shows absorbance at 211 nm (λ_1) and 280 nm (λ_2) corresponding to the absorbance maxima of saxagliptin and methyldopa respectively. In absorbance ratio method (Method II) isobestic point is observed at 240 nm. Isobestic point (240 nm) is considered as λ_1 and absorbance maxima of methyldopa (280 nm) is considered as λ_2. In area under curve method (Method III) measurement of area under curve in the range of 204-241nm (for saxagliptin) and 265-314 nm (for methyldopa) was carried out. In dual wavelength method (Method IV), saxagliptin and methyldopa were quantified using principle that absorbance difference between two points on mixture spectra was directly proportional to concentration of component of interest and independent of interfering component. All dilutions were prepared in distilled water. Linearity range was observed in the concentration range of solution 5-30 µg/ml for saxagliptin and 2-12 µg/ml for methyldopa. The methods were validated statistically and recovery study was performed to confirm the accuracy of both drugs.

Key words: Saxagliptin, Methyldopa, Ultraviolet spectroscopy, Simultaneous estimation, Absorbance ratio method, Dual wavelength method, Area under curve method.

INTRODUCTION

Saxagliptin, (1S, 3S, 5S)-2-[(2S)-2-amino-2-(3-hydroxyadamantan-1-yl)acetyl]-2-azabicyclo[3.1.0] hexane - 3-carbonitrile) is used as monotherapy and in combination regimens, wich has been associated with significant reductions in HbA1c and significant increases in the rate of achieving target HbA1c in patients with type-2 diabetes mellitus (T2DM). It has been reported to be well tolerated compared with other oral antihyperglycemic agents.

Methyldopa (L-α-Methyl-3, 4-dihydroxy phenyl alanine.) is an alpha-adrenergic agonist (selective for α_2-adrenergic receptors) psychoactive drug used as a sym patholytic or antihypertensive. Its use is now mostly deprecated following the introduction of alternative safer classes of agents. However, it continues to have a role in otherwise difficult to treat hypertension and gestational hypertension (previously known as pregnancy-induced hypertension (PIH). A diabetic hypertensive pregnant woman is being treated with saxagliptin and methyldopa. Various method for have been reported for estimation of saxagliptin and methyldopa separately. But no method for simultaneous estimation of saxagliptin and methyldopa is available. In present work we propose to develop and validate UV methods for simultaneous estimation of saxagliptin and methyldopa [1-6].

MATERIALS AND METHODS

Instrumentation

UV-visible double beam spectrophotometer, Jasco model 630 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells was used.

Reagent and chemicals

Standard gift sample of methyldopa and saxagliptin was obtaind from Wockhardt Pvt. Ltd. The synthetic mixture was prepared by using standard active pharmaceutical ingredient (saxagliptin and methyldopa).

Corresponding Author :- S.**M. Mhaske** Email:- mhaskesagar2@gmail.com

Asian J. Pharm. Res. Vol 4, Issue 3, 134-140, 2014.

After assessing the solubility of drugs in different solvents distilled water was used as common solvent for developing spectral characteristics.

Preparation of standard stock solution

The standard stock solutions (100µg/ml) of each of saxagliptin and methyldopa were prepared separately by dissolving accurately about 10 mg of drug in 20 ml of distilled water and volume was made up to 100 ml with distilled water producing working standard solutions of 100 µg/ml concentration.

Preparation of calibration curves

Solutions of 10 µg/ml of saxagliptin and methyldopa each were prepared separately. Both the solutions were scanned in the spectrum mode from 200-400 nm. The maximum absorbance of saxagliptin and methyldopa were observed at 211 nm and 280 nm, respectively. Saxagliptin and methyldopa obey Beer-Lamberts law in the concentration range of 5-30 µg/ml and 2-12 µg/ml at their respective maxima. Accurately measured standard stock solution of saxagliptin (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ml) and methyldopa (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 ml) were transferred to a separate series of 10 ml of volumetric flasks and diluted to the mark with distilled water. The absorbance of each solution was measured at wavelength 211 nm and 280 nm for saxagliptin and methyldopa respectively. The coefficient of correlation was found to be 0.999 and 0.999 for saxagliptin and methyldopa respectively. Results are as shown in Figures 1-6.

Method I. Simultaneous Equation Method

Sample stock solution was appropriately diluted with distilled water to obtain final concentration of 10 µg/ml for saxagliptin and methyldopa. These solutions were scanned in the wavelength range of 200 – 400 nm. From the overlain spectrum, two wavelengths namely 211 nm and 280 nm, λ-max of saxagliptin and methyldopa respectively were selected. The calibration curves were constructed in the concentration range of 5-30 µg/ml for saxagliptin and methyldopa. The concentration of drugs was determined by using the equation no. 1 and 2 [7, 8]. Results are show in table no.6.

$C_x = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2$ Equation no. 1

$C_y = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2$ Equation no.2

Where, A_1 and A_2 are absorbance of sample at 211 nm and 280 nm respectively; ax_1 and ax_2 are absorptivities of saxagliptin at 211 nm and 280 nm respectively; ay_1 and ay_2 are absorptivities of methyldopa at 211 nm and 280 nm respectively; C_x and C_y are concentrations of and saxagliptin and methyldopa respectively.

Method II. Absorbance Ratio Method

Two wavelengths were selected, from the overlain spectrum of saxagliptin and methyldopa: λ_1 observed at 240 nm which is the isoabsorptive point (Figure 7) for both drugs and λ_2 is considered at 280 nm which is λ max of methyldopa. The absorbance of the sample solutions were measured in a similar manner as described in the previous experiment. Wavelengths of absorptions were measured and the absorbance ratio values for both drugs at selected wavelengths were also calculated. The method employs Q-values and the concentration levels of drugs in sample solution were determined by using the equation no. 3 and 4 [9, 10]. Results are show in table no.6.

$C_x = Q_m - Q_y / Q_x - Q_y \times A/ax_1$ Equation no.2

$C_y = Q_m - Q_x / Q_x - Q_y \times A/ay_1$ Equation no.3

$Q_m = A_2/A_1$

$Q_x = ax_2/ax_1$, $Q_y = ay_2/ay_1$

C_x and C_y are concentrations of saxagliptin and methyldopa respectively; Q_x = the ratio of absorptivity of saxagliptin at 240 and 280 nm; Q_y = the ratio of absorptivity of methyldopa at 240 and 280 nm; Q_m = the ratio of absorbance of mixture at 240 and 280 nm; A = the absorbance of mixture at isoabsorptive point; ax = the absorptivity value of saxagliptin at isoabsorptive point; ay = the absorptivity value of methyldopa at isoabsorptive point.

Method III. Area under Curve Method

For the simultaneous determination using the area under curve method, suitable dilutions of the standard stock solutions (100µg/ml) of saxagliptin and methyldopa were prepared separately in distilled water. The solutions of drugs were scanned in the range of 200 nm - 400 nm. For area under curve method, calibration curve was plotted and the sampling wavelength ranges selected for estimation of saxagliptin and methyldopa are 204 nm -241 nm (λ_1-λ_2) and 265-314 nm (λ_3-λ_4) respectively (Figure 8 and 9) and area were integrated between these selected wavelength ranges for both drugs, which showed linear response with increasing concentration hence the same wavelength range were used for estimation of synthetic mixture. By using integrated areas two simultaneous equations were constructed and solved to determine concentrations of analytes. Concentration of two drugs in mixed standard and the sample solution were calculated using equation no. 5 and 6 [11]. Results are show in table no.6.

$Csaxagliptin = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2$ Equation no.5

$Cmethyldopa = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2$ Equation no.6

Where, ax_1 and ax_2 are absorptivities of SAXA at (λ_1-λ_2) and (λ_3-λ_4) respectively. ay_1 and $ay_2$are absorptivities of MD at (λ_1-λ_2) and (λ_3-λ_4) respectively. A_1 and A_2 are Absorbance of mixed standard at (λ_1-λ_2) and

Asian J. Pharm. Res. Vol 4, Issue 3, 134-140, 2014.

(λ_3-λ_4) respectively. Csaxagliptin and Cmethyldopa are the concentrations in µg /100 ml.

Method IV. Dual Wavelength Method

In this method, for the determination of methyldopa, the difference in absorbance at 206 nm and 216 nm (difference was zero for saxagliptin) was plotted against the concentration of methyldopa (Figure 10). saxagliptin was determined by plotting the difference in absorbance at 276 nm and 291 nm (difference was zero for methyldopa) against the concentration of saxagliptin (Figure 11). Similarly standard solutions were prepared having concentration 5-30 µg/ml for saxagliptin and 2-12 µg/ml for methyldopa. The difference in absorbance 276 nm and 291 nm were plotted against the concentration of saxagliptin and that at 208 nm and 216 nm were plotted against the concentration of methyldopa to construct two separate calibration curves for methyldopa and saxagliptin [12]. Results are show in table no.3, 4, 5.

Analysis of laboratory mixture

A bulk mixture of both drugs (SAXA and MD) was prepared using 10 mg of SAXA and 10 mg of MD. Common excipients which are used in tablet formulation were added in this laboratory mixture, triturated well and weighed. This powder was transferred to 100 ml of volumetric flask, dissolved in sufficient quantity of distilled water and volume was adjusted up to the mark with distilled water. The sample solution thus prepared was filtered through Whatman filter paper no. 44, diluted with distilled water to get the solution containing about 10 µg/ml of SAXA and 10 µg/ml of MD. A UV spectrum is as shown in Figure 12.

Validation of Method

Validation of the proposed methods was carried out for its accuracy, precision, specificity and linearity according to ICH guidelines.

Accuracy

Recovery studies were carried out at 80%, 100% and 120% level by adding a known quantity of pure drug to the preanalyzed laboratory mixture and the proposed method was followed. From the amount of drug found, percentage recovery was calculated and results are given in Table 1.

Repeatability

Six test sample solutions containing 10 µg/mL of SAXA and 10 µg/mL of DM were scanned over range of 200-400 nm and absorbance are measured at 211 nm and 280 nm respectively, concentrations were determined with the help of proposed method and % RSD was calculated and results are given in Table 2.

Limit of detection

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the equation no.7 and shown in Table 6.

$$LOD = 3.3 (\sigma / S) \qquad \text{Equation no.7}$$

Limit of quantification

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the equation no.8 and shown in Table 6.

Where, S = slope of calibration curve**,** σ = standard deviation of the response.

m= Slope, c= Intercepts

$$LOQ = 10 (\sigma / S) \qquad \text{Equation no.8}$$

Table 1. Recovery studies

S No.	Initial conc. (µg/mL)		Conc. of excess drug added to analyte (µg/mL)		Conc. found (µg/mL)		% Recovery		% RSD	
	SAXA	***MD***	***SAXA***	***MD***	***SAXA***	***MD***	***SAXA***	***MD***	***SAXA***	***MD***
1.	10	10	8	8	17.94	17.83	99.33	99.05	0.918	0.311
2.	10	10	10	10	19.93	19.89	99.3	99.45	0.755	1.8
3.	10	10	12	12	21.91	22.01	99.24	100.04	0.885	1.21

Table 2. Repeatability

S. No.	Concentration found (µg/mL)	
	SAXA	***DM***
1.	9.34	9.54
2.	9.67	10.09
3.	10.4	9.78
4.	9.91	9.23

5.	9.75	9.93
6.	10.03	9.67
Mean	9.85	9.7066
% RSD	0.3580	0.3028

Table 3. Determination methyldopa in presence of Saxagliptin

Series B			Series C		
Composition of mixture (µg/ml)		Difference in Absorbance (λ 276 – λ 291)	Synthetic mixture (µg/ml)		Difference in absorbance (λ 276 – λ 291)
Methyldopa	Saxagliptin		Methyldopa	Saxagliptin	
0	5	0.070	2	5	0.6912
0	10	0.1269	4	10	0.1301
0	15	0.1753	6	15	0.1502
0	20	0.2410	8	20	0.2399
0	25	0.3011	10	25	0.2975
0	30	0.3577	12	30	0.3595

Table 4. Determination of Saxagliptin in presence of Methyldopa

Series A			Series C		
Composition of mixture (µg/ml)		Difference in Absorbance (λ 208 – λ 216)	Synthetic mixture (µg/ml)		Difference in absorbance (λ 208 – λ 216)
Methyldopa	Saxagliptin		Methyldopa	Saxagliptin	
2	0	0.2083	2	5	0.1983
4	0	0.2937	4	10	0.2731
6	0	0.3885	6	15	0.3991
8	0	0.4632	8	20	0.4536
10	0	0.5503	10	25	0.5332
12	0	0.6119	12	30	0.6415

Table 5. Regression analysis data of the calibration curve obtained using series A, B, C

Series	Composition of the sample solution		Regression equation of the curve	Coefficient of correlation (R^2)
	Methyldopa (µg/ml)	Saxagliptin (µg/ml)		
A	2-12	0	Y=0.032X-0.009	0.999
B	0	5-30	Y=0.027X+0.008	0.999
C	2-12	5-30	*Y=0.040X+0.133	0.996
			**Y=0.011X+0.009	0.998

*equation of line for Saxagliptin
**equation of line for Methyldopa

Table 6. Optical characteristics, Precision study and result of formulation analysis

Parameter		Saxagliptin			Methyldopa		
		Method 1	Method 2	Method 3	Method 1	Method 2	Method 3
Wavelength (nm)		211	240 & 280	204-241	280	240 & 280	265-314
Beer's law limit (µg/mL)		5-30	5 - 30	5-30	2-12	2-12	2-12
Regression Eqn*	(m)	0.027	0.027	0.135	0.032	0.032	0.227
	(c)	0.008	0.008	0.306	-0.009	-0.009	0.326
Correlation coefficient (r)		0.999	0.999	0.999	0.999	0.999	0.999
Formulation Analysis (% Assay)		100.8	100.3	100.6	101.65	99.89	100.3
LOD (µg/mL)		0.267	0.261	0.241	0.275	0.262	0.243
LOQ (µg/mL)		0.812	0.792	0.789	0.836	0.798	0.894

Asian J. Pharm. Res. Vol 4, Issue 3, 134-140, 2014.

Figure 1. UV spectrum of saxagliptin (10µg/ml)

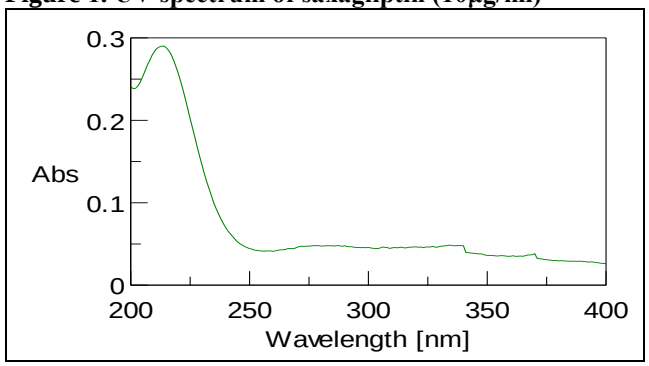


Figure 2. UV spectrum of methyldopa

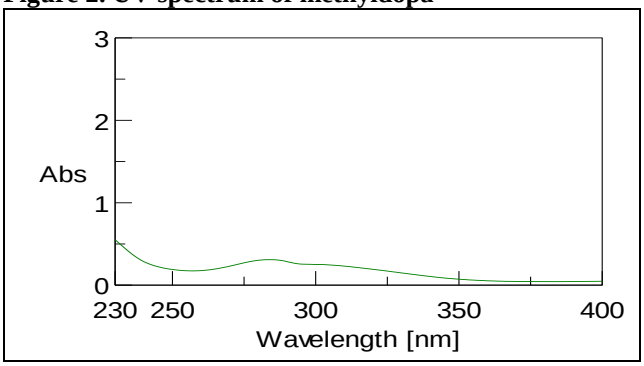


Figure 3. Overlay spectrum of saxagliptin (5 – 30 µg/mL)

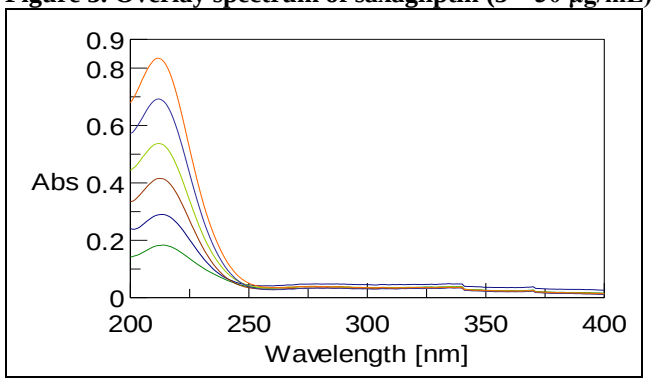


Figure 4. Linearity of Saxagliptin (5 – 30 µg/mL)

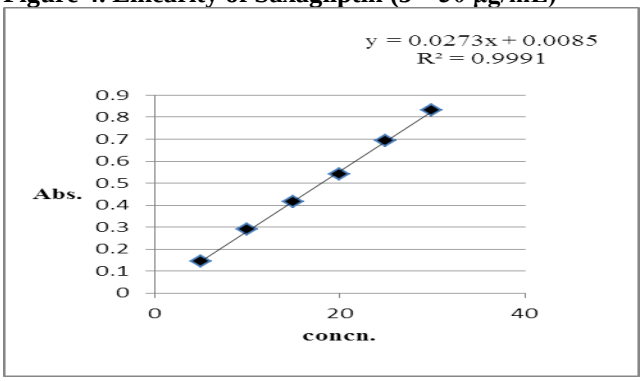


Figure 5. Overlay spectra of methyldopa (2-12 µg/mL)

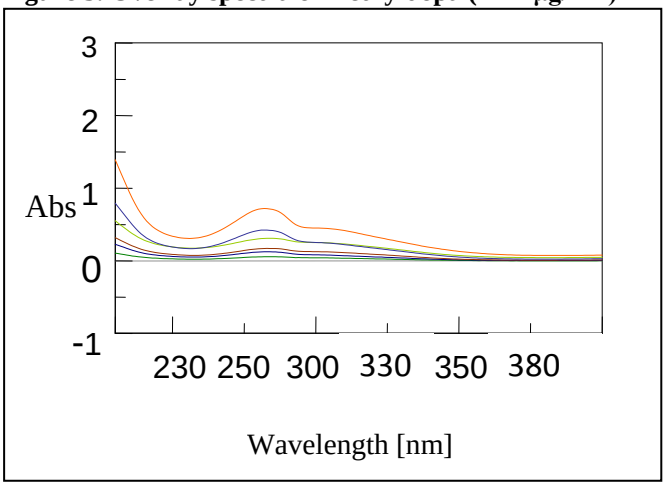


Figure 6. Linearity of Methyldopa (2-12 µg/mL)

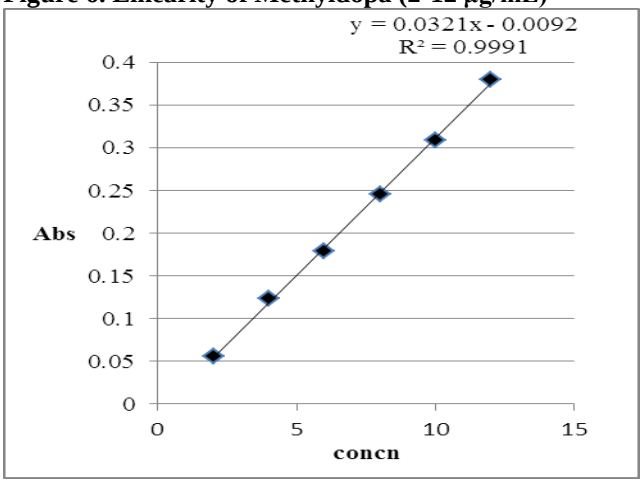


Figure 7. Isoabsorptive point

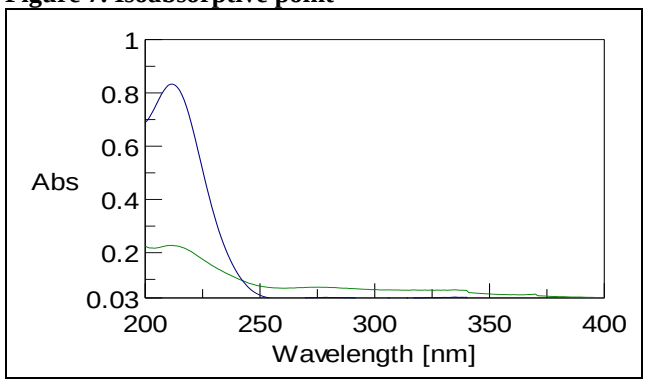
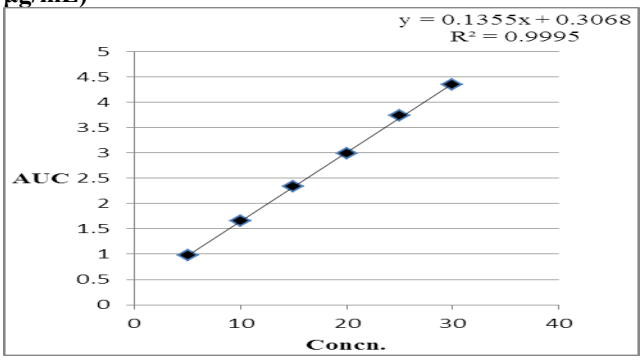


Figure 8. AUC of Saxagliptin at 204 nm -241 nm (5 – 30 µg/mL)



Asian J. Pharm. Res. Vol 4, Issue 3, 134-140, 2014.

Figure 9. AUC of Methyldopa at 265nm-314 nm (2-12 µg/mL)

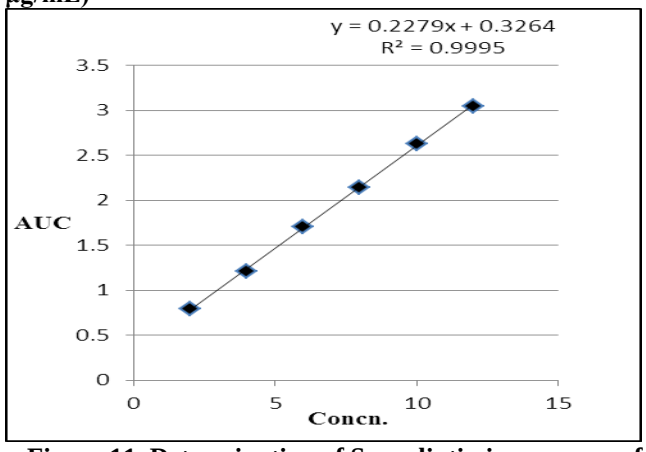


Figure 10. Determination of Methyldopa in presence of Saxagliptin (206 nm and 216 nm)

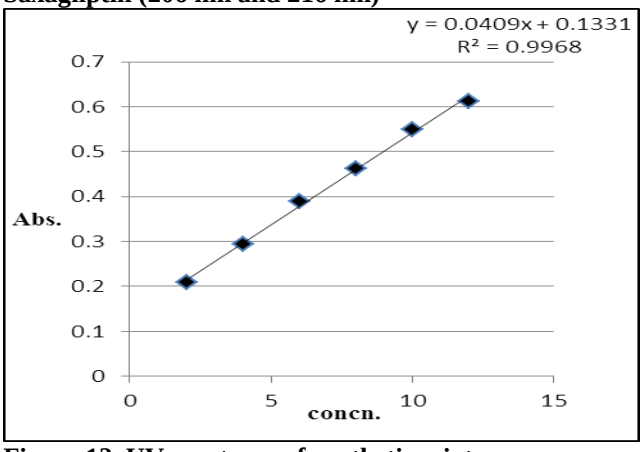


Figure 11. Determination of Saxagliptin in presence of Methyldopa (276 nm and 291 nm)

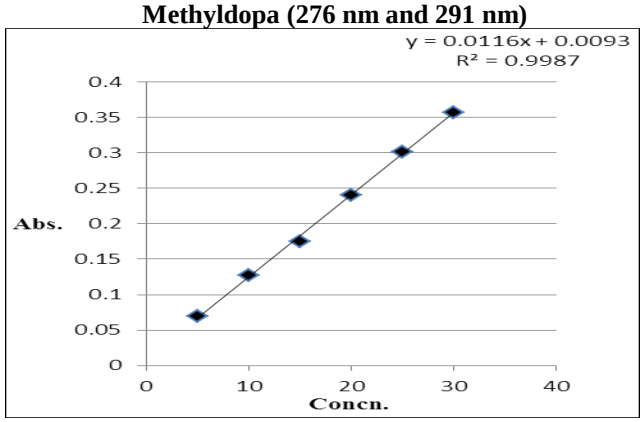
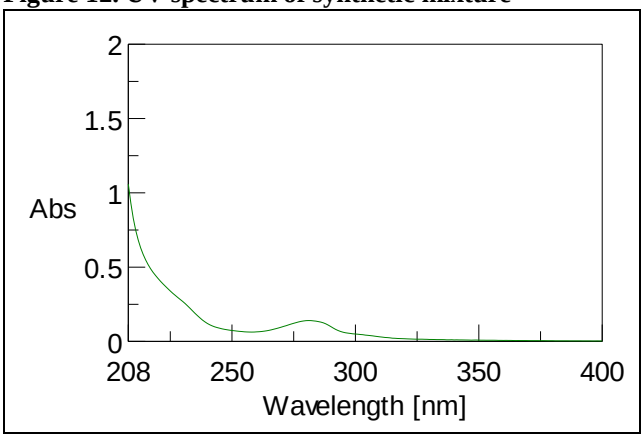


Figure 12. UV spectrum of synthetic mixture



RESULT AND DISCUSSION

Saxagliptin and methyldopa has estimated at 211 nm and 280 nm in distilled water, saxagliptin and methyldopa obey Beer-Lamberts law in concentration range of 5-30 µg/ml and 2-12 µg/ml respectively. The method was validated as per ICH and USP guidelines. The result of recovery study was found to be within the prescribed limit of 98 – 101 %. The assay results obtained by proposed methods are as shown in Table 6. The % R.S.D. Linearity was observed by linear regression equation method for saxagliptin and methyldopa in different concentration range. The correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity, hence it can be used for routine analysis of two drugs in combined dosage forms. There was no interference from tablet excipients was observed in these methods. These methods were accurate, simple, rapid, precise, reliable, sensitive, reproducible and economic.

CONCLUSION

The proposed methods are simple, precise, and accurate and can be used for routine quantitative analysis of saxagliptin and methyldopa in pure and synthetic mixture form.

REFERENCES

1. Deanna S, Gonzalvo J, Weber Z. Saxagliptin: A Clinical Review in the Treatment of type 2 Diabetes Mellitus. *Clinical Therapeutics*, 33, 2011, 1005-1022.
2. Tripathi KD, Essentials of medical pharmacology, 6th ed., Jaypee brothers medical publishers, New Delhi 2010, 540,547, 553.
3. Anonymous 1.Drugbank Saxagliptin (DB06335) http://www.drugbank.com/drugs/DB06335 (16 SEP. 2013).
4. Anonymous 2.Drugbank Methyldopa (DB00968) http://www.drugbank.com/drugs/DB00968 (12 JAN. 2014).
5. Nyola N, Jeyabalan G. Development and validation of UV-Vis spectroscopy method for simultaneous estimation of saxagliptin hydrochloride and metformin hydrochloride in active pharmaceutical ingredient. *Journal of Pharmaceutical Education Research*, 3(2), 2012.

6. Gadkariem E, Ibrahim K. A new spectrophotometric method for the determination of methyldopa. *Saudi Pharmaceutical Journal*, 17(4), 2009, 289-293.
7. The Indian Pharmacopoeia, Govt. of India, Ministry of Health and Family Welfare Controller of Publication, 2007, III, 750-751.
8. Beckett AH, Stenlake JB, Practical Pharmaceutical Chemistry. 4th ed., CBS Publisher and Distributors, New Delhi, Part-II, 278-282, 286-290.
9. Fernandes N, Nimdeo M, Choudhari V, Kulkarni R, Pande V, Nikalje A. Dual wavelength and simultaneous equation spectrophotometric methods for estimation of atenolol and indapamide in their combined dosage form. *International Journal of Chemical Sciences*, 6(1), 2008, 29-35.
10. Khalid M, Younis A, Younis M. Development of UV spectrophotometric method for the simultaneous estimation of ibuprofen and famotidine in tablet dosage form by absorbance correction method and absorbance ratio method. *International Journal of Research in Pharmacy and Science*, 2(3), 2012, 69-80.
11. Rana S, Pandya J, Solanki S, Patel M. Development and validation of spectrophotometric method for simultaneous estimation of lafutidine and domperidone in combined dosage form by area under curve method. *International Journal of Drug Development & Research*, 4(1), 2012, 257-262.
12. Bharat G, Chaudhari. Development and validation of dual wavelength spectrophotometric method for simultaneous estimation of amlodipine besylate and enalapril maleate in combined dosage form. *International Journal of Pharmaceutical Chemical and Biological Sciences*, 2(4), 2012, 626-632.